APPENDIX I

163. A method of treating tissue to prevent or control air or fluid leaks comprising:

providing a composition to tissue, said composition including a[n] ***serum*** albumin protein ***at about 20-60 wt/vol %*** and a crosslinking agent ***at about 50-800 mg/ml***, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein ***and having a molecular weight in a range of about 1,000-15,000***; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix ***that has a burst strength greater than about 10 mm Hg.***

19 ~~164.~~ 18 The method of claim ~~163~~ wherein said composition is cured to produce the matrix in less than about 10 minutes.

20 ~~165.~~ 18 The method of claim ~~163~~ wherein said composition is cured to produce the matrix in less than about one minute.

21 ~~166.~~ 18 The method of claim ~~163~~ wherein said composition is cured to produce the matrix in about ten seconds.

22 ~~167.~~ 18 The method of claim ~~163~~ comprising providing the composition to the tissue using a syringe.

23 ~~168.~~ 18 The method of claim ~~163~~ comprising providing the composition to the tissue using a dual syringe.

24 ~~169.~~ 18 The method of claim ~~163~~ comprising providing the composition to the tissue using a spray apparatus.

25 ~~170.~~ 18 The method of claim ~~163~~ wherein the matrix is resorbed.

26 ~~171.~~ 25 The method of claim ~~170~~ wherein the matrix is resorbed in about four to sixty days.

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27 18
172. The method of claim 163 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

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173. The method of claim 163 wherein the matrix has a burst pressure of about 34 mmHg or greater.

29 28
174. The method of claim 173 wherein the matrix has a burst pressure of about 90 mmHg or greater.

30 29
175. The method of claim 174 wherein the matrix has a burst pressure of about 130 mmHg or greater.

31 18
176. The method of claim 163 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

32 18
177. The method of claim 163 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

33 32
178. The method of claim 177 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

34 18
179. The method of claim 163 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

180. The method of claim 179 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

$-O-(CH_2-CH_2-O-)_a-$

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, -$(CH_2)_bC(O)$- where b is an integer from 1-5, a diester radical of the formula, -C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula -C(O)-O-$(CH_2)_d$-O-C(O)- where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, -R-C(O)-$(CH_2)_c$-C(O)-, or -R-C(O)-O-$(CH_2)_d$-O- where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

181. The method of claim 180 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

182. The method of claim 181 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

183. The method of claim 180 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

184. The method of claim 180 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

185. The method of claim 180 wherein -LM- is an oligomeric diradical -R-C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

186. The method of claim 180 wherein -G is succinimidyl.

~~187~~ 42 The method of claim ~~180~~ 35 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

~~188~~ 43 The method of claim ~~180~~ 35 wherein -LM- is a diester diradical of the formula -C(O)-$(CH_2)_2$-C(O)-.

~~189~~ 44 The method of claim ~~180~~ 35 wherein -LM- is a diester diradical of the formula -C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

~~190~~ 45 The method of claim ~~180~~ 35 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

~~191~~ 46 The method of claim ~~165~~ 18 comprising treating tissue to prevent or control a fluid leak.

~~192~~ 47 The method of claim ~~191~~ 46 wherein the fluid leak is a blood leak.

~~193~~ 48 The method of claim ~~165~~ 18 wherein the tissue includes an air leak.

194. The method of claim ***193*** wherein the air leak is in the pulmonary system.

195. A method of treating tissue to prevent formation of an adhesion comprising:

providing a composition to tissue, said composition including a[n] ***serum*** albumin protein ***at about 20-60 wt/vol %*** and a crosslinking agent ***of about 50-800 mg/ml***, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein ***and having a molecular weight in the range of about 1,000-15,000***; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix ***that has a burst strength greater than about 10 mm Hg***.

51 50
196. The method of claim 195 wherein said composition is cured to produce the matrix in less than about 10 minutes.

52 50
197. The method of claim 195 wherein said composition is cured to produce the matrix in less than about one minute.

53 50
198. The method of claim 195 wherein said composition is cured to produce the matrix in about ten seconds.

54 50
199. The method of claim 195 comprising providing the composition to the tissue using a syringe.

55 50
200. The method of claim 195 comprising providing the composition to the tissue using a dual syringe.

56 50
201. The method of claim 195 comprising providing the composition to the tissue using a spray apparatus.

57 50
202. The method of claim 195 wherein the matrix is resorbed.

58 51
203. The method of claim 202 wherein the matrix is resorbed in about four to sixty days.

59 50
204. The method of claim 195 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

60 50
205. The method of claim 195 wherein the matrix has a burst pressure of about 34 mmHg or greater.

206. The method of claim 2053 wherein the matrix has a burst pressure of about 90 mmHg or greater.

62 61
207. The method of claim 206 wherein the matrix has a burst pressure of about 130 mmHg or greater.

63 50

208. The method of claim 195 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

64 50

209. The method of claim 195 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

65 64

210. The method of claim 209 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

66 50

211. The method of claim 195 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

212. The method of claim 211 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

$-O-(CH_2-CH_2-O-)_a-$

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, $-(CH_2)_bC(O)-$ where b is an integer from 1-5, a diester radical of the formula, $-C(O)-(CH_2)_c-C(O)-$ where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula $-C(O)-O-(CH_2)_d-O-C(O)-$ where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, $-R-C(O)-(CH_2)_c-C(O)-$, or $-R-C(O)-O-(CH_2)_d-O-$ where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

68 67
213. The method of claim 212 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

69 68
214. The method of claim 213 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

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215. The method of claim 212 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

71 67
216. The method of claim 212 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

72 67
217. The method of claim 212 wherein -LM- is an oligomeric diradical -R-C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

73 67
218. The method of claim 212 wherein -G is succinimidyl.

74 67
219. The method of claim 212 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

75 67
220. The method of claim 212 wherein -LM- is a diester diradical of the formula -C(O)-$(CH_2)_2$-C(O)-.

76 67
221. The method of claim 212 wherein -LM- is a diester diradical of the formula -C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

77 67
222. The method of claim 212 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

223. The method of claim 195 wherein the composition is provided to tissue at a surgical site.

224. The method of claim 195 wherein the composition is provided on a surface of an internal organ.

225. A method of treating tissue to bind layers of tissue together comprising:

providing a composition to tissue, said composition including a[n] ***serum*** albumin protein ***at about 20-60 wt/vol %*** and a crosslinking agent ***at about 50-800 mg/ml***, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein ***and having a molecular weight in the range of about 1000-15,000***; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix ***that has a burst strength of greater than about 10 mm Hg***.

226. The method of claim 225 wherein said composition is cured to produce the matrix in less than about 10 minutes.

227. The method of claim 225 wherein said composition is cured to produce the matrix in less than about one minute.

228. The method of claim 225 wherein said composition is cured to produce the matrix in about ten seconds.

229. The method of claim 225 comprising providing the composition to the tissue using a syringe.

230. The method of claim 225 comprising providing the composition to the tissue using a dual syringe.

231. The method of claim 225 comprising providing the composition to the tissue using a spray apparatus.

232. The method of claim 225 wherein the matrix is resorbed.

88 87
233. The method of claim 232 wherein the matrix is resorbed in about four to sixty days.

89 80
234. The method of claim 225 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

90 80
235. The method of claim 225 wherein the matrix has a burst pressure of about 34 mmHg or greater.

91 90
236. The method of claim 235 wherein the matrix has a burst pressure of about 90 mmHg or greater.

92 91
237. The method of claim 236 wherein the matrix has a burst pressure of about 130 mmHg or greater.

93 80
238. The method of claim 225 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

94 80
239. The method of claim 225 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

95 99 94
240. The method of claim 239 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

96 80
241. The method of claim 225 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

242. The method of claim 241 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

$-O-(CH_2-CH_2-O-)_a-$

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, $-(CH_2)_bC(O)-$ where b is an integer from 1-5, a diester radical of the formula, $-C(O)-(CH_2)_c-C(O)-$ where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula $-C(O)-O-(CH_2)_d-O-C(O)-$ where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, $-R-C(O)-(CH_2)_c-C(O)-$, or $-R-C(O)-O-(CH_2)_d-O-$ where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

~~243~~ 98 97. The method of claim ~~242~~ 242 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

~~244~~ 99 98. The method of claim ~~243~~ 243 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

~~245~~ 100 97. The method of claim ~~242~~ 242 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

~~246~~ 101 97. The method of claim ~~242~~ 242 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

~~247~~ 102 97. The method of claim ~~242~~ 242 wherein -LM- is an oligomeric diradical -R-C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

~~248~~ 103 97. The method of claim ~~242~~ 242 wherein -G is succinimidyl.

249. The method of claim 242 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

250. The method of claim 242 wherein -LM- is a diester diradical of the formula $-C(O)-(CH_2)_2-C(O)-$.

251. The method of claim 242 wherein -LM- is a diester diradical of the formula $-C(O)-(CH_2)_c-C(O)-$ where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

252. The method of claim 242 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

253. The method of claim 225 wherein the matrix binds tissue together in addition to a suture, a staple, a tape, or a bandage.

254. The method of claim 225 wherein the composition is provided to attach skin grafts.

255. The method of claim 225 wherein the composition is provided to attach adjacent layers of tissue.

256. The method of claim 225 wherein the composition is provided to position tissue flaps.

257. The method of claim 225 wherein the composition is provided to close gingival flaps.

258. A method of treating tissue comprising:

providing a composition to tissue, said composition including a[n] ***serum*** albumin protein ***at about 20-60 wt/vol%*** and a crosslinking agent ***at about 50-800 mg/ml***, said crosslinking agent having a polyoxyethylene chain portion and an activated leaving group which allows the crosslinking agent to react with said protein ***and having a molecular weight in a range of about 1000-15,000***; and

curing said composition on the tissue to bond said composition to the tissue and to provide a substantive cured matrix ***that has a burst strength greater than about 10 mm Hg.***

114 113
259. The method of claim 258 wherein said composition is cured to produce the matrix in less than about 10 minutes.

115 113
260. The method of claim 258 wherein said composition is cured to produce the matrix in less than about one minute.

116 113
261. The method of claim 258 wherein said composition is cured to produce the matrix in about ten seconds.

B1 cont.

117 113
262. The method of claim 258 comprising providing the composition to the tissue using a syringe.

118 113
263. The method of claim 258 comprising providing the composition to the tissue using a dual syringe.

119 113
264. The method of claim 258 comprising providing the composition to the tissue using a spray apparatus.

120 113
265. The method of claim 258 wherein the matrix is resorbed.

121 120
266. The method of claim 265 wherein the matrix is resorbed in about four to sixty days.

122 113
267. The method of claim 258 comprising curing the composition such that the peel strength of the matrix is about 0.08 lb/in or more.

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268. The method of claim 225 wherein the matrix has a burst pressure of about 34 mmHg or greater.

269. The method of claim 226 wherein the matrix has a burst pressure of about 90 mmHg or greater.

34

270. The method of claim 236 wherein the matrix has a burst pressure of about 130 mmHg or greater.

271. The method of claim 258 comprising providing a composition wherein the crosslinking agent has a molecular weight in a range of about 1,000-5,000.

272. The method of claim 258 comprising providing a composition wherein the activated leaving group is an N-hydroxy imide.

273. The method of claim 272 comprising providing a composition wherein the activated leaving group is N-hydroxy succinimide.

274. The method of claim 258 further comprising mixing a first mixture and a second mixture to form the composition and applying said composition to the tissue,

wherein the first mixture includes about 20-60 wt/vol% of the protein in about 0.01-0.25 molar buffer at a pH in a range of about 8.0-11.0 and the second mixture includes about 50-800 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-15,000.

275. The method of claim 274 wherein the crosslinking agent is of the formula

G-LM-PEG-LM-G

wherein:

-PEG- is a diradical fragment represented by the formula

$-O-(CH_2-CH_2-O-)_a-$

where a is an integer from 20-300;

-LM- is a diradical fragment selected from the group consisting of a carbonate diradical of the formula, -C(O)-, a monoester diradical of the formula, $-(CH_2)_bC(O)-$ where b is an integer from 1-5, a diester radical of the formula, $-C(O)-(CH_2)_c-C(O)-$ where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated, and a dicarbonate diradical of the formula $-C(O)-O-(CH_2)_d-O-C(O)-$ where d is an integer from 2-10, or an oligomeric diradical represented by the formulas -R-C(O)-, $-R-C(O)-(CH_2)_c-C(O)-$, or $-R-C(O)-O-(CH_2)_d-O-$ where c is an integer from 2-10, d is an integer from 2-10, and R is a polymer or copolymer having 1-

10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone; and

-G is the leaving group selected from the group consisting of succinimidyl, maleimidyl, phthalimidyl, imidazolyl, nitrophenyl, or tresyl.

131 130
276. The method of claim 275 wherein the protein in the first mixture is about 35-45 wt/vol% serum albumin.

132 131
277. The method of claim 276 wherein the buffer is 0.05-0.15 molar carbonate/bicarbonate buffer at a pH of about 9.0-10.5.

B1 D21 278. The method of claim 275 wherein the second mixture is about 5-300 mg/ml of the crosslinking agent having a molecular weight in a range of about 1,000-5,000.

cont 134 130
279. The method of claim 275 wherein the ratio of a volume of the first mixture to a volume of the second mixture is in a range of about 1:10 to about 10:1.

135 130
280. The method of claim 275 wherein -LM- is an oligomeric diradical -R-C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and R is a polymer or copolymer having 1-10 monomeric fragments selected from the group consisting of lactide, glycolide, trimethylene carbonate, caprolactone, and p-dioxanone.

136 130
281. The method of claim 275 wherein -G is succinimidyl.

137 130
282. The method of claim 275 wherein the second mixture includes about 300-800 mg/ml of a crosslinking agent having a molecular weight in a range of about 5,000-15,000.

138 130
283. The method of claim 275 wherein -LM- is a diester diradical of the formula -C(O)-$(CH_2)_2$-C(O)-.

139 130
284. The method of claim 275 wherein -LM- is a diester diradical of the formula -C(O)-$(CH_2)_c$-C(O)- where c is an integer from 2-10 and where the aliphatic portion of the diradical may be saturated or unsaturated.

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140 130
285. The method of claim 275 wherein -LM- is an oligomeric diradical derived from polyglycolic acid.

141 113
286. The method of claim 258 comprising curing the composition on the tissue to seal the tissue.

142 141
287. The method of claim 286 comprising treating tissue to prevent or control a fluid leak.

143 142
288. The method of claim 287 wherein the fluid leak is a blood leak.

144 141
289. The method of claim 286 wherein the tissue includes an air leak.

B1 Cont. claims 022

290. The method of claim 289 wherein the air leak is in the pulmonary system.

146 113
291. The method of claim 258 wherein the composition is provided to tissue at a surgical site.

147 113
292. The method of claims 258 comprising curing the composition at the tissue to prevent a tissue adhesion.

148 113
293. The method of claim 258 wherein the composition is provided on a surface of an internal organ.

149 113
294. The method of claim 258 comprising curing the composition to form a matrix to bind tissue.

150 149
295. The method of claim 294 wherein the matrix binds tissue together in addition to a suture, a staple, a tape, or a bandage.

151 113
296. The method of claim 258 wherein the composition is provided to attach skin grafts.

152 113
297. The method of claim 258 wherein the composition is provided to attach adjacent layers of tissue.

37

153 113

~~298.~~ The method of claim ~~258~~ wherein the composition is provided to position tissue flaps.

154 113

~~299.~~ The method of claim ~~258~~ wherein the composition is provided to close gingival flaps.

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